

What is claimed is:

*Sub 2* 1. A method for measuring protease which comprises the steps of:

- (1) bringing a sample containing protease into contact with a thin membrane which comprises a protease substrate together with a hardening agent and is formed on a surface of a support; and
- (2) detecting the trace of digestion formed on the thin membrane by the action of protease.

2. A method for measuring protease which comprises the steps of:

- (1) bringing one of two substantially continuous slices of a biological sample into contact with a thin membrane which comprises a protease substrate together with a hardening agent and is formed on a surface of a support;
- (2) detecting the trace of digestion formed on the thin membrane by the action of protease; and
- (3) comparing the trace of digestion with a histopathological preparation prepared from the other slice.

3. A method for measuring protease which comprises the steps of:

- (1) bringing one of two or more substantially continuous slices of a biological sample into contact with a thin membrane which comprises a protease substrate together with a hardening agent and is formed on a surface of a support;
- (2) bringing the remaining slices into contact with a thin membrane which comprises a protease substrate, a hardening agent, and a protease inhibitor and is formed on a surface of a support;
- (3) detecting traces of digestion formed on the thin membranes by the action of protease; and
- (4) comparing the trace of digestion on the thin membrane used in the step (1) with the trace of digestion on the thin membrane used in the step (2).

4. A method for measuring protease which comprises the steps of:

- (1) bringing one of two or more substantially continuous slices of a biological sample into contact with a thin membrane which comprises a protease substrate together with a hardening agent and is formed on a surface of a support;
- (2) bringing the remaining slices into contact with a thin membrane which comprises a

protease substrate different from the protease substrate contained in the thin membrane used in the step (1) together with a hardening agent and is formed on a surface of a support;

(3) detecting traces of digestion formed on the thin membranes by the action of protease; and

(4) comparing the trace of digestion on the thin membrane used in the step (1) with the trace of digestion on the thin membrane used in the step (2).

5. A method for measuring protease which comprises the steps of:

(1) bringing a sample containing protease into contact with a thin membrane which comprises at least the following two layers: layer (a) which contains a protease substrate, a hardening agent, and a protease inhibitor and is formed on a surface of a support, and layer (b) which contains a protease substrate and a hardening agent and is laminated on the layer (a);

(2) detecting traces of digestion formed on the thin membrane by the action of protease; and

(3) comparing the trace of digestion on the layer (a) with the trace of digestion on the layer (b).

6. A method for measuring protease which comprises the steps of:

(1) bringing a sample containing protease into contact with a thin membrane which comprises at least the following two layers: layer (a) which contains a protease substrate together with a hardening agent and is formed on a surface of a support, and layer (b) which contains a protease substrate different from the protease substrate contained in the layer (a) together with a hardening agent and is laminated on the layer (a);

(2) detecting traces of digestion formed on the thin membrane by the action of protease; and

(3) comparing the trace of digestion on the layer (a) with the trace of digestion on the layer (b).

7. The method of any one of claims 1 to 6 wherein the protease substrate is selected from the group consisting of collagen, gelatin, proteoglycan, fibronectin, laminin, elastin, and casein.

8. The method of any one of claims 1 to 7 wherein the sample is a biological

sample isolated or collected from a patient.

9. The method of any one of claims 1 to 8 wherein the detection is performed by using a thin membrane containing one or more substances selected from the group consisting of metals, metal oxides, pigments and dyes and having a maximum transmission density of 0.01 or higher at a wavelength ranging from 400 nm to 700 nm.

10. The method of any one of claims 1 to 9 wherein the protease is matrix metalloproteinase.

11. A thin membrane for measuring protease which contains a protease substrate together with a hardening agent and is formed on a surface of a support.

12. The thin membrane of claim 11 which comprises at least the following two layers: layer (a) which comprises a protease substrate, a hardening agent and a protease inhibitor and is formed on a surface of a support, and layer (b) which contains a protease substrate together with a hardening agent and is laminated on the layer (a).

13. The thin membrane of claim 11 which comprises at least the following two layers: layer (a) which comprises a protease substrate together with a hardening agent and is formed on a surface of a support, and layer (b) which comprises a protease substrate different from the protease substrate contained in the layer (a) together with a hardening agent and is laminated on the layer (a).

14. The thin membrane of any one of claims 11 to 13 which comprise one or more substances selected from the group consisting of metals, metal oxides, pigments and dyes and have a maximum transmission density of 0.01 or higher at a wavelength ranging from 400 nm to 700 nm;

15. The thin membrane of any one of claims 11 to 14 wherein the support is selected from a microscope slide and polyethylene terephthalate film.

16. The thin membrane of any one of claims 11 to 15 wherein an undercoat layer is provided between the support and the thin membrane.

17. A method of diagnosing a disease involving protease which comprises steps of:

- (1) bringing a biological sample isolated or collected from a patient into contact with a thin membrane which comprises a protease substrate together with a hardening agent and is formed on a surface of a support; and
- (2) detecting the trace of digestion formed on the thin membrane by the action of

protease.

18. The method of 17 wherein the disease is selected from the group consisting of cancer, rheumatic diseases, periodontal diseases and alveolar pyorrhea.